STUDY OF BLOOD AND TISSUE EOSINOPHILS IN PARASITISED AND NON-PARASITISED DOGS

by

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INTRODUCTION

The eosinophilic leukocyte has received a tremendous amount of attention since its first description by T. W. Jones in 1846 and its characterization and naming by Ehrlich in 1879. The conspicuousness of the cell due to its granules and its association with several disease conditions in both man and animals have attracted the attention of many investigators. The body of literature on the eosinophilic leukocyte is enormous but what has truly been established with certainty can be summarized in just a few pages. The functions of the eosinophil in the body have not been fully established to this present day. The role of the cell in allergic and parasitic diseases is currently becoming understood, however the association of the cell with other disease states cannot yet be explained (73). Diagnostic procedures for the various disease conditions associated with eosinophilia have low reliability due to the apparent erratic nature of the cell in the body with regards to its distribution in the blood and the tissues. Conflicting views are still being held on the validity of certain laboratory findings by several investigators working with different animal species and man. The objective of this investigation is to identify the quantitative relationship between blood and tissue eosinophils with special reference to sampling time in parasitised and non-parasitised dogs and to find whether the differences in these two groups of dogs could find quantitative clinical application that could be employed to diagnose various disease conditions associated with cell. The findings may further provide

a method of rational interpretation of laboratory results in such disease states and also provide a means of monitoring the course of the various disease conditions associated with the cell.

LITERATURE REVIEW

Brief Historical Review

T. W. Jones in 1846 first described the eosinophilic leukocyte in his report to the Royal Society. In 1879 Ehrlich noted the affinity of the coarse eosinophil cytoplasmic granules for acid dyes and named the cell Eosinophil, after the acid dye eosin (4, 20, 28). Tremendous amounts of literature have accumulated since the discovery of the cell, mostly directed at elucidating the functions of this cell. Cline (28) cited the work of Schwartz in 1914 in which 2,758 references were given on the eosinophilic leukocyte. Braunsteiner and Zucker-Franklin (20) had provided in table form "The developments concern with the eosinophils up to 1914." Several other investigators had directed their efforts towards elucidating the association of the cell with certain disease conditions and to further explain the role that the cell plays in these states. Despite these efforts, the functions of the cell still remain an enigma.

Origin and Development of the Eosinophil

The eosinophilic leukocyte has been identified in several tissues and organs of the body. Among the other tissues and organs, the cell has been identified in the lymph nodes and the thymus gland. The thymus in children has been shown to contain large number of eosinophils. During fetal life, production of the eosinophil has been assigned primarily to the lymph nodes and the thymus (70, 126). After fetal life, however, the production of the cell is restricted almost

exclusively to the bone marrow, in most mammalian species. In rodents however there had been some speculations that the cell could be produced in the spleen also. Current findings have confirmed that in this species, only maturation takes place in the spleen. The finding is consistent with other findings which reported that unlike in other mammalian species, the mature eosinophil pool in the rodents constitutes only 10% of the total eosinophil population in the bone marrow. The bone marrow has been established to be the predominant site of eosinophil production in the mammalian species (20, 28, 31, 70, 111, 132).

The various cell lines in the granuloyctic series follow the same pattern of proliferation, differentiation, maturation and storage in the bone marrow. There has been some controversies concerning the origin of the stem cell for the eosinophilic, neutrophilic and the basophilic series. Some have contended that there is a common stem cell that produces all the cells in the granulocytic series. Others have stated that there are specific stem cells for each of the cell lines in the bone marrow. Support for this latter stand has emanated from the observations that some patients suffering from a granulocytosis with marked neutropenia may still have normal or even greatly elevated eosinophil counts. The enzymes and spectral properties of eosinophil peroxidase were found to differ from neutrophil myeloperoxidase; also, a familial absence of eosinophil peroxidase has been reported in the presence of normal neutorphil myeloperoxidase activities (28, 136). These findings suggest that each cell line in the bone marrow has its own specific stem cell. The shortcoming of this conclusion lies in the fact that if mutation took place at the promyelocyte or the myelocyte stage, then the various

cell lines could still originate from a common stem cell (28, 31, 111, 132).

With Romanovsky-stained bone marrow preparation, the eosinophilic promyelocyte is the earliest identifiable cell of this series. Such a cell may contain 10-12 small eosinophilic granules admixed with other azurophilic granules. The promyelocyte is relatively more difficult to identify in normal bone marrow preparations (28, 131, 132). The prominence of the eosinophil cytoplasmic granules increases with further development and by the time the myelocyte stage is reached, the azurophilic granules may no longer present (28). The eosinophilic myelocyte is the least mature cell of this series commonly encountered in bone marrow examinations. The eosinophilic metamyelocyte and band forms are smaller than the myelocyte and contain fewer eosinophilic granules in their cytoplasm.

The same characteristics employed to determine the stage of maturity of other cells in the granulocytic series are utilized for the eosinophilic leukocyte. This includes the degree of condensation of the nuclear chromation, the presence of nucleoli and the characteristics of the cytoplasmic granules (3, 28, 131, 132). Consistent with the findings in the other granulocytic series, the young eosinophilic granulocyte has high nuclear to cytoplasmic ratio with nuclei characterised by diffuse chromatin with a well developed nucleoli. As these cells mature further, the nuclear chromatin becomes more condensed and this is accompanied by reduction in the number and prominence of the nucleoli.

Functions of the Eosinophil

Despite the tremendous amount of research efforts on the eosinophilic

leukocyte, its primary function in the body remains virtually unknown. As pointed out earlier there have been some advances in the areas of immediate-type hypersensitivity reactions and parasitosis, however the role of the cell in the other disease conditions still remains an enigma (3, 4, 9, 12, 26, 33, 49, 53, 56, 61, 73, 77, 81, 91, 93, 95, 99, 107, 111, 113, 129, 137).

Both in vitro and in vivo studies on the eosinophilic leukocyte, aimed at elucidating the functions of the cell, have been extensively studied. While in vitro systems are more amenable to control, findings may not necessarily represent the actual state of affairs in the intact animal. Advances have been made in different areas of study of the cell.

Chemotaxis

Considerations for chemotaxis stems from the fact that the eosinophils are attracted from the peripheral blood into the various body tissues by substances released into these tissues in response to variable number of stimuli. The eosinophils at these tissue sites are believed to serve the role of inactivating the substances that attracted them to the sites in the first place, as well as playing other roles incompletely understood at this time. These chemotactic substances could be antigen-antibody complexes and other mediators such as histamine released in the inflammatory reactions (56).

The eosinophilic leukocyte has been observed to exhibit ameboid motions in culture media and migrates towards certain chemical substances. In vivo, the cell has been reported to accumulate in small numbers at the sites of acute inflammations. The neutrophils on the other hand were present in larger numbers

at these inflammatory sites. The eosinophils accumulate in larger numbers at the sites of chronic inflammatory reactions (28, 70). Cline (28) reported that localised antigen within the walls of the gastrointestinal tract and other tissues attracts eosinophils to the site for periods up to 10 months. In vivo and in vitro studies have demonstrated that several chemical agents generated within the body have chemotactic activities for both neutrophils and the eosinophils, while others show relative specificity for eosinophil attraction. The products of complement activation, C3a, C5a and the trimolecular complex $C\overline{567}$, have been shown to have chemotactic activities for the eosinophils as well as the neutrophils (43, 71, 79, 105, 132). Lachmann (79) conducted an investigation in which the trimolecular complex of complement $C\overline{567}$ was prepared by interaction of purified $C\overline{56}$ and C7 solutions. He was able to demonstrate the chemotactic activity of this $C\overline{567}$ complex for both the neutrophils and the eosinophils. He further re-reported that this chemotactic effect was not affected by the presence of C8 and C9 even when these were present in slight excess. When the interaction of $C\overline{56}$ with C7 took place in the presence of an excess of erythrocytes, the cells bound the $C\overline{567}$ complexes and no chemotactic effect was observed. Ward (128) also demonstrated the chemotactic activities of $C\overline{567}$ for the neutrophils and the eosinophils. He explained the requirement of antigen-antibody complexes for the activation of serum complement. Kay (71) demonstrated the chemotactic activities of eosinophil chemotactic factor of anaphylaxis (ECF-A) and the fragment (C5a) cleaved from the fifth component of complement, for both the neutrophils and the eosinophils. He reported that the chemotactic effect was the same

for both these cells, however when the eosinophil population comprise more than 10% of the total leukocyte population, there was a preferential attraction of the eosinophils by both ECF-A and C5a.

Eosinophil chemotactic factor of anaphylaxis (ECF-A) is one of the mediators of inflammation released from mast cells and/or basophils in tissues sensitised by lgE antibodies. Mast cells sensitised by IgE antibodies release ECF-A when stimulated by an appropriate antigen (56). ECF-A has been prepared and purified by challenge of guinea-pig lung tissue with suitable antigen (26). In the rat, the mast cell has been established to be the source of ECF-A and it has been localised within the granules of this cell (95). ECF-A has also been characterised and its molecular weight has been estimated at approximately 5,000 daltons. ECF-A has also been distinguished from other small molecules such as slow reacting substance of anaphylaxis (SRS-A), serotonin, brady kinin, prostaglandins and others. These latter chemical substances were found not to have any chemotactic properties for either eosinophils or the neutrophils. ECF-A is preformed in the mast cell and its release requires divalent cations, intact glycolytic metabolic pathway and diisopropyl fluorophosphate inhibitable esterase (56). This latter article reported that ECF-A may play a significant role in the localisation of the eosinophils within the tissues, at the sites of inflammation. ECF-A was also reported to act synergistically with C5a.

There seems to be some controversy over the chemotactic properties of histamine for the eosinophils. Archer (11) clearly demonstrated that histamine was chemotactic for the eosinophils in the horse, through intradermal injection

of this substance. The accumulation of the eosinophils at the injection site suggested the chemotactic property of histamine for this cell. Other workers however were unable to reproduce Archer's experiment in the other species. Zucker-Franklin (137) has reported one such finding in an experiment in which histamine failed to attract eosinophils to the site of intradermal injection, in species other than the horse. This had led the author to the conclusion that the chemotactic effect of histamine was species dependent. Similar experiment in the human revealed that the chemotactic effect of histamine could only be demonstrated in atopic subjects. Parish (99) failed to produce peripheral blood eosinophilia in guinea-pigs and rats by local injection of histamine, however there was a local accumulation of the eosinophils at the injection site. Recent report indicated that the chemotactic properties of histamine for the eosinophils depend on the dosage as well as the route of administration. Perhaps the failure to reproduce Archer's experiment may be due to these factors in the different species tested (56, 73, 85, 95, 111, 137). Mast cell degranulation released chemical mediators including histamine. Tissues rich in mast cells such as the lungs and the gastrointestinal tract have high incidence of eosinophils (56, 73).

Antigen-antibody complexes have been shown to possess chemotactic properties for the eosinophils. Schalm (111) reported that the eosinophil has been assigned the function of inactivating these antigen-antibody complexes. Antigen-antibody complexes particularly when combined with activated components of complement have been reported by several investigators to have chemotactic effect for both the eosinophils and the neutrophils (3, 45, 56, 63, 72, 99, 111,

128, 137). Some of these workers have reported that antigen-antibody complexes have chemotactic effect for the eosinophils on their own. Others have speculated that these complexes exert their chemotactic effects through activation of the complement system. Cline (28) reported on an experiment by Litt who demonstrated phagocytosis of immune complexes by the eosinophils in the guinea-pig. In this study labelled antibody to bovine serum albumen (BSA) with green fluorescent dye and labelled bovine albumen with a red fluorescent dye were injected intraperitoneally in the guinea-pigs. The peripheral blood eosinophils were attracted by these products into the peritoneal cavity and the eosinophils fluoresced with the complementary color, yellow, thus indicating phagocytosis of the immune complexes by the eosinophils as well as the possible chemotactic effect of these complexes. Ishikawa (63) also demonstrated the uptake of antigen-antibody complexes by eosinophils. He reported that in vitro soluble antigen-antibody complexes containing ragweed antigen and human IgM or IgG antibodies were more frequently taken up by the eosinophils than complexes containing IgA. This finding then confirmed the views of several other investigators who believed that only antigen-antibody complexes in which the component antibody was of the IgG or the IgM class were more capable of attracting the eosinophils and the neutrophils. Others have reported that immune complexes containing IgE, prepared with extreme or moderate antigen excess were more frequently taken up by the eosinophils than complexes containing IgG. Zolov et al (134) conducted an experiment to investigate the correlation between blood eosinophil levels and the antibody classes in penicillin-hypersensitive human patients. His findings indicated that in these

patients, penicillin induced eosinophilia correlated with the presence of skin sensitizing antibodies (SSA orI gE) and not with IgG or IgM. Takenaka (123) also found in another investigation that eosinophilia was not necessarily accompanied by increase in serum IgE levels.

Cline (28) reported that most antigens alone have little or no chemotactic properties for the eosinophils. The same was also true with antibody alone. Litt (84) reported that the non-precipitating antigen-antibody complexes are less effective as chemotactic agents. The mechanism by which antigen-antibody complexes induce eosinophil migration can only be speculated: (a) chemotactic factors could be released from the antigen-antibody complexes (3, 4); (b) chemotactic factors may be released by delayed type hypersensitivity reactions in which mononuclear cells play a significant role (28); (c) chemotactic factors could be released from fibrin formation or other large proteins (28).

The role of the lymphokines in eosinophil chemotoxis has been identified recently. Eosinophil stimulating promoter (ESP) is a lymphokine produced by stimulated lymphocytes on contact with antigens or mitogens. ESP has been thoroughly investigated and has been found to be specifically chemotactic for the eosinophils (30, 45, 46, 65, 102, 129). The discovery of this soluble mediator, chemotactic for the eosinophils thus linked the mechanism of eosinophilia with the immune system (117).

Cline (28) cited works which reported that eosinophils have been observed to be attracted towards macrophages and actually penetrate these cells. The eosinophils were observed to be mainly attracted toward damaged macrophages,

into which they penetrate and were subsequently phagocytised by other macrophages. In vivo, the eosinophils have been observed to form a rosette around macrophages primed with antigen. It was thus suggested that these antigen-primed macrophages may release chemotactic substances upon second exposure to antigen.

In summary, it could be stated that eosinophils in general respond to chemotactic stimuli to which the neutrophils are also capable of responding. The identification of substances such as ECF-A and ESP which have some specificity for eosinophil attraction seems to provide a clue on the mechanism of the cell accumulation in the various inflammatory reactions. As was reported earlier, the eosinophils in acute inflammatory reactions were present only in fewer numbers relative to the neutrophils and that their numbers at these inflammatory sites increase as these processes turn chonic. This then suggests the presence of specific chemotactic agents for the eosinophils and the neutorphils (128).

Phagocytosis

Phagocytosis has been observed in the eosinophil since 1915. It was then suggested that the eosinophils were attracted by antigen-antibody complexes which they eventually phogocytise and inactivate. The eosinophils are capable of ingesting a variety of substances including inert materials. The cells have been observed to ingest immune complexes, zymosan, antibody coated red blood cells, mycoplasmas, Esherichia coli, Staphylococcus aureus and other bacteria, fungi and mast cell granules. These findings have been reported in both in vitro and in vivo systems (3, 4, 20, 26, 28, 63, 73, 99, 111, 137). The significance of phagocytosis

in the eosinophil has however not been established. Phagocytosis by the neutrophil has been thoroughly studied by Thomas Stossel (117, 118, 119). The process in the neutrophil does not differ from that observed in eosinophil. The eosinophils were reported to be more sluggish in their phagocytic activities compared to the neutrophils. Cline (28) incubated eosinophils and neutrophils with Staphylococcus aureus at a ratio of several bacteria to one leukocyte. He observed that the neutrophils quickly became engorged with the bacteria while the eosinophils ingested only a few. Phagocytosis by eosinophils, as observed by several investigators cannot be regarded as signifying an equivalent function in the neutrophils and monocytes. As mentioned earlier the process in both the eosinophil and the neutrophil is essentially the same. Both phagocytise substances and degranulate in the same manner, both physically and metabolically. The oxidative response to phagocytosis by the eosinophil is greater than that of the neutrophil (73). This signifies larger production of hydrogen peroxide in the eosinophil compared to the neutrophil. In spite of this finding, the eosinophil has been reported to have no bactericidal activities. Eosinophil peroxidase has no bactericidal properties comparable to that of the neutrophil myeloperoxidase (117, 118, 119). The most current information on the bactericidal properties of the eosinophil has been reported by Migler et al (190). They reported on the bactericidal activities of human eosinophil peroxidase enzyme. They found that the peroxidase enzyme in both neutrophils and possibly eosinophils has its bactericidal properties through either the decarboxylation of amino acids in the presence of chloride or iodination of proteins in the presence of iodide. These

activities were then examined in sonicated extracts of purified neutrophils and eosinophils and were then related to the bactericidal abilities of these cells. They found that unlike the neutrophil, the eosinophil cell-free extracts were unable to decarboxylate L-alanine-1-^{14}C. Iodination was measured by the ability of peroxidase to convert ^{125}I into trichloracetic acid-precipitable form. The result indicated that the iodination was greater in the resting eosinophil than in the neutrophil; however, both of these cells showed increased iodination upon ingestion of zymosan. When the iodide was used as the halide, eosinophil and neutrophil cell-free preparations showed bactericidal activity as measured by the reduction in the viability of Staphylococcus aureus and Esherichia coli. When the chloride was used as the halide, only neutrophils could effectively kill these bacteria. The findings were in agreement with earlier observations which reported that eosinophil peroxidase was unable to catalyse decarboxylation reactions. This then suggests the importance of this latter mechanism in the bactericidal activities of the cells. The findings in this investigation call for more study on the possible bactericidal role of the eosinophil.

Role of the Eosinophil in Parasitic Infestations

The association of peripheral blood eosinophilia with intestinal helminthiasis, especially those with migratory larval stages, has long been observed in both clinical and experimental situations (4, 12, 20, 26, 28, 49, 87, 111). Ascaris lumbricoides, Toxocara canis, hookworms of dogs and cats, have been known to produce eosinophilia in man, dog and the cat. Taenia saginata and Taenia solium produce

low grade peripheral blood eosinophilia in man. Peripheral blood eosinophilia has been produced experimentally in rats through intravenous injection of Trichinella spiralis (26). The production of eosinophilia has been demonstrated to require normal bone marrow and lymphocytes. Eosinophilia of the peripheral blood has also been produced in x-irradiated rats through infusion of thoracic duct lymphocytes that were sensitised to Trichirella spiralis antigens. This further demonstrated the requirement of lymphocytes for the production of peripheral blood eosinophilia.

Kay (73, 1976) has given a comprehensive review of current research directed toward elucidation of the role of the eosinophil in the body including its possible role in parasitic diseases. He reported that normal leukocytes, normal in the sense that they have not been sensitised to an antigen, have the ability to lyse antibody-coated target cells. The affector cell in most systems has been shown to be a sub-population of lymphocytes. The reaction has been termed, lymphocyte-dependent-antibody mediated cytotoxicity (LDAC) or K-cell (Killer) cytolysis. In some systems, the neutrophil or macrophage have been identified as the affector cells and all require antibody coated target cells with intact Fc region. In vitro studies on human peripheral blood leukocytes were shown to release chromium from labelled immature schistosomes. When such leukocytes were separated into eosinophil and neutrophil cell rich fractions, K-cell activities were found associated with the eosinophil rich fraction. Further investigation by the same author found that patients with peripheral blood eosinophilia had eosinophils that were unable to exhibit the K-cell activities. The explanation

given was that the lack of K-cell activity in the eosinophils could be attributed to an interference with cell surface receptors by circulating antigen-antibody complexes.

Mahmoud et al (87) in their classic investigations showed further evidence to support the effector or K-cell role played by the eosinophilic leukocyte, in antibody-dependent damage to schistosoma mansoni, in vivo. These workers were able to demonstrate that in schistosome infections in mice, partial immunity could be transferred by immune serum which contains specific antibodies directed against the schistosomes. The protection provided by this antiserum was lost by prior treatment of the mice with anti-eosinophil serum but not with antisera directed against the lymphocytes, monocytes or neutrophils. This then has given a clear indication of the role of the eosinophil in parasitic infestation, in this study. It is not known whether this experimental finding could be extended to other parasitic disease conditions.

Eosinophils in Inflammation

The role of the eosinophil in immediate-type hypersensitivity reaction has been thoroughly investigated in the human (56). The association of eosinophilia with this type of reaction has led to the conclusion that the eosinophil plays the role of inactivation of pharmacologically active mediators liberated in these reactions in both animals and man. These mediators include: histamine and eosinophil chemotactic factor of anaphylaxis (ECF-A) which are preformed in mast cells and/or basophils; slow reacting substance of anaphylaxis (SRS-A), serotonin (5-hydroxytryptamine), bradykinin and other Kinins, prostaglandins

and platelet inactivating factor (PAF) (4, 28, 56, 111, 121). There are great species variations in the clinical and pathological reactions which follow challenge of an actively sensitized animal with a given antigen. This may be due to differences in the response to the immunogen as well as to certain unique anatomical or pharmacological characteristics of the species (121).

lgE antibody has been reported to sensitize target cells and these, on contact with a suitable antigen, release pharmacological mediators which exert their effect on target cells elsewhere in the body as well as attracting eosinophils to these inflammatory sites (56, 121, 123). The principal role of the eosinophil at these inflammatory sites is that of inactivation of the pharmacologic mediators. Histamine inactivation by the eosinophils has been demonstrated by Archer (4) in his classical work on the horse. He reported in vitro inactivation of histamine by the eosinophils but could not explain the mechanism of the reaction. In another investigation, Archer reported that aqueous extracts of horse eosinophils tested in vivo against histamine, had reduced antihistaminic effect. The same finding was also reported in guinea-pig eosinophil extract. The author attributed these findings to faulty technique in preparing the eosinophil extracts. Cline (28) reported chemical inactivation of histamine by the eosinophils and suggested that the mechanism of the reaction may involve amine oxidation.

Hubschner (56) has provided a recent report on the role of the eosinophil in immediate-type hypersensitivity reactions. He reported on the release of a substance from the eosinophil called eosinophil-derived inhibitor (EDI) of histamine release which was capable of inhibiting further histamine release

from human mast cell and the basophil, by acting through the cyclic AMP system. EDI is a low molecular weight substance which has been reported to consist of a mixture of prostaglandins E_1 and E_2 (56, 73). Hubschner (56) has reported on the role of EDI in a "feed-back loop" leading to the inhibition of mediator release at the inflammatory sites. His findings indicated that there was an initial immunologic reaction associated with inflammations in which lgE bound mast cells react with antigen leading to decrease in intra-cellular cyclic AMP and release of the mediators which react with receptors on the cells. There is an H-1 receptor for histamine on the target cells and an H-2 receptor on mast cells. Reaction of histamine with the H-2 receptors of the mast cell results in increase in intracellular cyclic AMP which inhibits further histamine release. The EDI elaborated by the eosinophils at these inflammatory sites reacts with other receptors on the mast cell to also stop further release of histamine. There are EDI receptors also in the bronchi and reaction with EDI has been reported to attenuate bronchospasm in the human.

The enzyme arylsulfatase, present in the small eosinophil granules, has been reported as serving the role of the inactivation of slow reacting substance of anaphylaxis (SRS-A). Histaminase role has been reported earlier. The bradykinin inactivating enzymes (kininases) of the eosinophils have also been mentioned elsewhere. Phospholipase D has been identified in the eosinophil and serves the role of inactivation of platelet activating factor (PAF) (56).

The relative importance of tissue stores of histaminase and arylsulfatase enzymes has not yet been established. These enzymes were found in tissues such

as the lung, skin, ileum, spleen and liver. It has been observed that in local anaphylactic responses, appreciable eosinophil infiltration of the tissues does not occur until several hours after antigen challenge, at a time when mediator release had almost certainly been terminated (73).

Eosinophil Kinetics

The bone marrow represents the major site of eosinophil production after fetal life in the mammalian species. These cells emanate from this site into the peripheral blood from where they are finally attracted into the various body tissues, where they are believed to serve their functions. It has been estimated that for every circulating eosinophil, there are approximately 300 in the bone marrow and an equivalent number in the other body tissues (26, 28, 111, 132).

Eosinophils spend 3-6 days in the bone marrow before they are released into the circulating blood. The half life of the eosinophil in the peripheral blood has been reported with considerable variability. Variations have been reported with animal species as well as the techniques employed in the studies. Wintrobe (132) reported that after continuous infusion of tritiated thymidine (^{3}HTdR) into rats, the half life of the eosinophils in the peripheral blood was estimated at 8-12 hours. After a single pulse infusion, however, the half life was found to be 6.7 hours in the peripheral blood. A half life of 4.5 hours has also been estimated for peripheral blood eosinophils in man. In the dog a much shorter half life has been reported. The half life of peripheral blood eosinophils in the dog has been found to be only 30 minutes (111).

Experimental evidence has indicated that the egress of the eosinophil from

the peripheral blood to the tissues does not depend on the age of the cell in the blood but occurs randomly (32, 98, 116, 132). Once the eosinophil leaves the peripheral blood, it probably never returns to this pool again (4, 28, 31, 111, 132, 137). Eosinophil kinetics has not been studied much due to the fact that the cells are normally present in low numbers in the peripheral blood and thus making it difficult to obtain pure fractions for the various studies. This has led to the study of the cell in disease states associated with eosinophilia, where adequate numbers of the cell are available. Dale et al (32) studied the eosinophil kinetics in six human patients with idiopathic hypereosinophilic syndrome. The blood eosinophils of these subjects in this study were labelled with chromium-^{51} in vitro. They found that these autologous labelled eosinophils transiently left the circulating blood in the first 3 hours after the infusion of the labelled eosinophils then re-entered the circulating pool again thereafter. This led the investigators to the conclusion that there has been a re-circulation of the eosinophils from the tissues to the circulating blood. The compartment into which the eosinophils were temporarily sequestered was however not identified. It was thus not known for certain whether the eosinophils had actually recirculated from the tissues to the peripheral blood in this study. Henrion, Glasser, Walker and Palmer (55) also studied the eosinophil kinetics in two patients with eosinophilia of non-leukemic origin. In this study, the eosinophils were also labelled with chromium-^{51} in vitro then re-injected into the subjects. They found that the leukocytes disappeared at different rates from the peripheral blood during the first 8 hours of the infusion. During the second 24 hours after the infusion, there was a significant

rise in peripheral blood radioactivity and this led to the conclusion that there has been a recirculation of the eosinophils. Again the compartment into which the cells temporarily went could not be identified.

After leaving the blood stream, the eosinophils are attracted in the various body tissues where they are believed to serve their functions. The peripheral blood may thus be looked upon as serving the role of a vehicle which merely transports these cells to their final destinations. The life span of the eosinophilic leukocyte in the body tissues has been reported to be much longer than that of the other leukocytes, in this compartment (21, 28, 31, 111, 132). Schalm (111) has reported that in culture media, the eosinophil life span was estimated to be 8-12 days. Burnet (21) had also reported the same life span of the eosinophils in culture media.

The fate of the eosinophil in the tissues has been a subject of much controversy over the years. Archer (4) has reviewed works done on this topic prior to this time (1963). The disappearance of the eosinophils from the peripheral blood in stressful conditions and following exogenous corticosteroid and ACTH administration has led some investigators to the conclusion that these chemical agents could lyse the eosinophils. Archer (4) set up an in vitro experiment in which he incubated eosinophils with corticosteroids and ACTH and reported that the cells were not lysed by these chemical agents. It was obvious that intravascular disintegration was not the usual fate of the eosinophil. Archer (4) also reported finding degenerative forms of the eosinophils in other body tissues, however he could not be certain whether these were the result of poor fixation

or represented the actual fate of the cell in those tissues. He suggested that when most of the eosinophils in the tissue sections are degenerative forms, and this could likely be attributed to poor fixation; however, when only a few degenerative forms are present, this may represent a significant finding. The author had identified few of these degenerative forms of the eosinophils in the tissues, but reported that their numbers were insignificant. He explained that these represent normal findings which have resulted from mechanical damage incurred during processing.

The reticuleoendothelial system has been reported to be the final destination of the eosinophils where they are phagocytised and destroyed by macrophages, after serving their functions in the various body tissues. Tissue eosinophilia was observed to accompany eosinophilia in the lymph nodes that drained these tissues and organs (4, 20, 124, 132). Archer (4) found only a few degenerative eosinophils in the lymph nodes of normal subjects and concluded that there was a rapid destruction of this cell in this organ. Other workers have reported that after serving their functions in the tissues, the eosinophils were finally shed into the lumen of the gastrointestinal and the respiratory tracts from where they were discharged from the body (20, 124).

Eosinophilia

Disease conditions associated with increased numbers of blood and tissue eosinophils are numerous. Prasse (104) and Cline (28) had provided a comprehensive list of disease conditions with eosinophilia in animals and man respectively. Experiments have been carried out to elucidate the mechanisms of eosinophilia

in both in vitro and in vivo systems. The findings in these experiments have been impressive in the areas of allergy and parasitosis (56, 73). Other disease conditions whose possible mechanisms cannot be speculated on the basis of established facts in other areas, have been classified as idiopathic hyper-eosinophilic syndromes (28, 32, 104, 132).

Archer (4) has formulated an hypothesis to explain the mechanism of eosinophilia. He investigated the role of histamine release in eosinophilia. He has earlier observed that tissue eosinophilia has a relationship with the mast cell content. Tissues rich in connective tissues, such as the lungs, gastro-intestinal tract and the urogenital tract were found to be rich in mast cells. Archer found that stress or injuries in the tissues rich in mast cells may lead to histamine release from such cells. Mechanical trauma including those caused by migrating helminth larva, bacterial substances, snake venom, heat, ultraviolet radiation and x-ray, have all been reported to promote histamine release and eosinophilia of the peripheral blood (54, 93, 104). Archer (4) stated that local histamine release in these stressed or injured tissues attract eosinophils to these sites. With the persistence of histamine release, blood concentration of this mediator rises and remains elevated as long as the stimulus persists. The persistent elevation of blood histamine concentration provides the primary stimulus for increased bone production and release of the eosinophils into the peripheral blood (4, 29). Histamine thus acts in two steps; first there is an initial local release which leads to local accumulation of eosinophils, accompanied by transient peripheral blood eosinopenia; secondly, further release of histamine leads to

elevation of the blood concentration of this mediator which in turn leads to the proliferation and release of eosinophils from the bone marrow to the peripheral blood. Williams (29) reported that the eosinopenic response in the peripheral blood following local injection of histamine occurred 2-4 hours after the injection. The bone marrow response was observed 24 hours later. Clark et al (26) reported on the induction of peripheral blood eosinophilia through intraperitoneal injection of histamine in the guinea-pig. The same author cited another experiment in which the later findings could not be reproduced in the guinea-pig. Another work cited by this author demonstrated that in the guinea-pig local injection of histamine did not produce local tissue eosinophilia at the injection site, however intravenous injection of the histamine produced blood eosinophilia. Archer (4) reported the opposite in the horse. His findings in this species indicated that intravenous administration of histamine produced eosinopenia but local tissue eosinophilia resulted with local injection of the histamine in the horse. Sampter (109) has reported on species variations in response to histamine. The role of histamine in eosinophilia of the peripheral blood is by no means the only mechanism. Other systems have been investigated as well in an attempt to answer some of the questions that have arisen with histamine experimentation.

The relationship between eosinophilia and the immune system has been well established through various experiments (10, 18, 22, 46, 49, 56, 65, 72, 87, 102, 128, 133, 134). The role of antigen-antibody complexes in eosinophil chemotaxis has been presented elsewhere. The production of peripheral blood eosinophilia through repeated injections of antigen has been reported. The role of

lymphocytes in the production of eosinophilia has also been mentioned earlier (10, 45, 46, 130). Walls et al (127) reported that the removal of the thymus gland in mice resulted in the inablility of these mice to respond to eosinophilic stimulation. This finding thus demonstrated the possible requirement of T-lymphocytes in the eosinophilic response. Warren (129) has also demonstrated that eosinophilia in human and murine Trichinella spiralis infections require the participation of the T-lymphocytes. The role of the eosinophil in passive immunity transferred with antisera directed against Schistosoma mansoni has been reported by Kay (73). The association of eosinophilia with antibody classes has been provided elsewhere (134). Grove (49) also conducted serological survey for antibody classes in a human population infested with hookworms. Similarly, Zucker-Franklin's work (137) on patients with agammaglobulinemia with respect to their response to eosinophilic stimulations has been reported. All these reports lend support for the requirement of the immune system for the production of blood eosinophilia in the various species. This and other findings on the mechanism of eosinophilia may thus represent an integrated working unit which functions harmoniously in the production of peripheral blood eosinophilia.

Eosinopenia

It has long been observed that stressful conditions and the acute stages of certain infectious diseases were characterised by decrease in the numbers of blood eosinophils in the human and animals (4, 8, 70, 92, 111, 132). These observations were attributed to the hyperactivities of the adrenal and the pituitary glands in these conditions. In the human with Addison's disease which is

characterised by adrenal cortical insufficiency eosinopenia is not seen even after test administration of ACTH (4). Experimentally, administration of adrenal corticosteroids and ACTH to humans and animals produced eosinopenia of the peripheral blood in the recipients. The Thorn test is based on the production of eosinopenia by adrenal corticosteroids. The test is used in humans to assess the functions of the adrenal cortex by administration of test dose of ACTH. Production of peripheral blood eosinopenia after this administration is indicative of a functional adrenal cortex (4, 111, 132). These findings have led to the search for the mechanisms by which adrenal corticosteroids and pituitary ACTH produce their eosinopenic effect.

Early speculation that adrenal corticosteroid and ACTH could lyse the eosinophils has been proved unlikely (4, 29, 111, 132). The requirement of the adrenal gland for the production of eosinopenia has been demonstrated. Archer (4) showed that adrenalectomy results in the loss of the eosinopenic effect following administration of ACTH to such animals. ACTH produces its eosinopenic effect by stimulating the adrenal cortex to secrete corticosteroids (132). It has been reported that the adrenal corticosteroids produce eosinopenia of the blood through enhancing the egress of the blood eosinophils to the tissues as well as inhibiting the release of the cells from the bone marrow (39). Wintrobe (132) reported that steroid eosinopenia occurs 2-3 hours following injection of the product. This has led him to the conclusion that inhibition of bone marrow release by the steroids alone cannot account for the eosinopenia, considering the survival time of blood eosinophils of 5-24 hours. The same author further observed that

continuous infusion of steroid will inhibit bone marrow release of the eosinophils as opposed to a single dose which has no such effect. Other reports indicated that after corticosteroid administration to animals, the tissue eosinophil numbers went up; thus giving support for steroid enhancement of the egress of the cell from the peripheral blood to the tissues (66). Archer (4) reported earlier that chronic infusion of histamine in animals produced eosinophilia. He thus theorized that corticosteroids and ACTH produced their eosinopenic effects through the suppression of histamine release. Schalm (111) supported this finding and further suggested the corticosteroids and ACTH suppresses histamine release by inhibiting mast cell regranulation, stabilizing the granule membrane of the mast cell and basophils and by interfering with the biogenesis of histamine. These together decrease blood histamine concentration and thus precipitate the blood eosinopenia (39, 111, 132).

Bass (8) studied the behavior of the eosinophilic leukocyte in acute inflammation. He observed that trichinosis in the mice was associated with eosinopenia of the peripheral blood. He found that in trichinosis in mice, the peak concentration of serum corticosterone was twice the concentration in the control group at the onset of clinical signs. He further measured serum corticosterone concentrations in experimentally produced *Esherichia coli* pyelonephritis in mice and found that the concentration was less than that observed in trichinosis at the peak concentration of corticosterone. In another experiment he infected mice with pneumococcus and produced eosinopenia but failed to demonstrate any rise in serum corticosterone. In yet another experiment Bass infected

adrenalectomised mice with pneumococcus and was able to observe peripheral blood eosinopenia. Based on these observations, he concluded that the eosinopenia of acute infections could not be attributed to adrenal cortical stimulation. This experiment was however not reproduced in other species.

Diurnal variations in peripheral eosinophil counts have been reported by several workers, with some variations. Wintrobe (132) reported high absolute eosinophil counts late at night and lower counts in the afternoons. In other works however, the same author reported that there was no fixed pattern common to all human subjects. Saran et al (110) conducted an investigation on the diurnal variations in the eosinophil counts in human patients with tropical eosinophilia. This is a disease condition caused by infection with Dirofilaria immitis. His findings were that the lowest absolute count was obtained in samples taken between midnight and 4 a.m. He supported the reliability of his findings by reasoning that his patients were more uniform at the time of sampling with respect to their physical and emotional activities. The same author also cited another work in this area in which the lowest count was recorded between 9-11 a.m. and the highest between midnight and 3 a.m. Halberg et al (51) reported that cyclic endogenous eosinopenia occurs in preparation for daily activities; in the morning eosinopenia occurs in the human and dogs and in the evenings, the same occurs in nocturnal animals such as rats, mice and hamsters.

Lee et al (80) reported "Circadian Rhythm in the Cutaneous reactivity to histamine" and its relationship to urinary cortisol excretion. Their findings indicated that the reduced histamine reactivity corresponds with low

urinary excretion of cortisol.

Halberg et al (51) investigated the role of light in the diurnal variations of eosinophil counts in the hamster. He subjected the animals to 12 hours of darkness alternated with 12 hours of light and found that the highest counts were obtained at noon while the lowest counts were recorded around midnight. The finding thus demonstrated internal timing of periodic eosinopenia. Pauly et al (101) observed that in the mouse, circadian fluctuation in the absolute eosinophil counts could be synchronized to the environmental light-dark cycle if food was always available.

Archer (4) reported on the role of epinephrine in eosinopenia. He found that the eosinopenic effect of epinephrine was species-dependent. He reported that eosinopenia could be produced in dogs and asthmatic patients with epinephrine injection. The same result was also obtained with the guinea-pig. In the horse, however, the author could not produce an eosinopenia of the peripheral blood even with a massive dose of epinephrine. The cow also failed to display the eosinopenic effect of epinephrine. Eosinopenia was produced in adrenalectomized and splenectomized rats with epinephrine. When the spleen was left intact in this latter experiment, eosinophilia was produced with the epinephrine injection. In mice, eosinopenia was produced with epinephrine, however, this could not be reproduced when the mice were splenectomized. Schalm (111) has reported on some of Archer's findings. Wintrobe (132) reported that there was an initial eosinophilia with epinephrine injection by the intra muscular route, in the human. The maximal eosinopenic effect of this injection was observed 17 minutes after

the administration. Subsequently the blood eosinophil counts returned to normal then finally went below the preinjection numbers. The explanation offered for this observation was that the initial eosinophilia was due to mobilization of the eosinophils in the marginated pool into the circulatory pool. The subsequent eosinopenia was attributed to the stimulation of ACTH production by the epinephrine. Sampter (121) also cited Wintrobe's findings and both have reported that epinephrine produces peripheral blood eosinopenia through mobilization of adrenal corticosteroids. Koch-Weser (76) also demonstrated the eosinopenic effect of epinephrine in the human. He further showed that this property could be abolished by pretreatment of the test subjects with propranolol (beta adrenergic drug). Whether this was the result of cell membrane or vascular-receptor inhibition, has not yet been established.

Distribution of Tissue Eosinophils

The distribution of the eosinophilic leukocyte in the body tissues and organs is quite extensive. It has been established that tissue eosinophils represent cells that have been attracted to these sites from the peripheral blood. This ends the earlier contention by some investigators that the tissue eosinophils represent developmental forms (20). The eosinophil concentration in tissues correlated with the mast cell content in these areas. Eosinophils have been identified in all the segments of the gastrointestinal tract and associated lymph nodes, the lungs, urogenital tract, the dermis of the skin, conjunctiva, thymus, pancreas and in other lymphoid tissues (4, 14, 15, 20, 84, 111, 124, 126). Vilpo (126) has presented in considerable detail, the distribution of eosinophils in

36 organs and tissues of the cow and pig.

Details of the eosinophil population in the bone marrow and peripheral blood has been provided elsewhere (31, 111, 132). The distribution of the cell in various other tissues and organs has not been adequately reported. Species differences have been reported in the tissue distribution of the eosinophilic leukocytes. The physiological state of the animal at the time of sampling may affect the distribution of the cells in the tissues. The distribution of the eosinophils within the gastrointestinal tract has been reported by several workers for various species including man and in both normal and disease states (14, 15, 17, 53, 54, 82, 83, 84, 126). Disease conditions such as eosinophilic gastroenteritis and ulcerative colitis in man and the dog were reported to have increased numbers of eosinophils in the various gastrointestinal segments. In normal subjects, the highest eosinophil concentration within the gastrointestinal tract has been observed in the human colon and a relatively lower incidence was found in the stomach and the small intestine. The distribution in the gastorintestinal tract was either diffuse or localised and extended through the entire length of the tract or was discontinuous. Parasitic diseases with migrating larval stages may result in localization in the walls of the gastrointestinal tract, leading to the formation of eosinophilic granuloma (20). Such antigen localization within the tissues, continuously attracts eosinophils to the site.

Cline (28) has reported that antigen localization within a tissue such as the intestine may continuously attract eosinophils to the site for periods up to 10 months. Vilpo (126) has also studied the distribution of the eosinophils within the

gastrointestinal tract of the cow and pig. He reported that the highest numbers of the eosinophil within the gastrointestinal tract were found in the walls of the small intestine and modest numbers were located in the stomach of these species. Schalm (111) reported that almost all the eosinophils in the walls of the gastro-intestinal tract were found in the lamina propria and the submucosa. He reported further that there were relatively fewer eosinophils in the submucosa of starved animals. Teir et al (124) has estimated that there are 50 million eosinophils per 100g body weight in the bowel of the rat. Archer (4) reported that only a few eosinophils were present in the gastrointestinal tract of normal man and animals and that the presence of more than 3 or 4 cells per high power field (x 400 magnification) should be regarded as signifying the presence of disease.

Attempts to correlate the numbers of eosinophils in the glandular stomach and the peripheral blood of animals have been unsuccessful. The same was also true with the other segments of the gastrointestinal tract (124).

Eosinophils in the lymph nodes are preferencially located in the cortical areas surrounding the lymphoid follicles and the trabeculae (50, 126). Litt (83, 84) found that lymph node eosinophils were located mainly in the cortex and the subcapsular sinuses. He also reported that few eosinophils were present in the germinal centers of the lymphoid follicles and in the medullary areas.

Eosinophils have been identified in the respiratory tract especially in allergic disorders (18, 56). In this organ, the cell has been found in the lung parenchyma and in the bronchi as well as other areas. Schalm (111) reported that in the bronchi the eosinophils were located mainly beneath the bronchial

epithelium.

Eosinophils in Disease

Eosinophilia is the hallmark of several disease states in both man and animals. Cline (28) has listed human diseases associated with eosinophilia. Some of these human diseases have their counterparts in the animals especially in dogs and cats. Prasse (104) has also listed disease conditions associated with eosinophilia in dogs and cats.

Eosinophilic Gastroenteritis

Eosinophilic gastroenteritis is one of the disease conditions common to both the dog and man. The disease is rare in both species. The disease in man has been accorded considerable attention since its discovery but the cause in the species still remained virtually unknown (1, 7, 33, 35, 48, 52, 53, 64, 67, 69, 78, 108). Evidences in man and the dog suggest an allergic basis of the disease. In man the disease is characterised by infiltration of the walls of the gastro-intestinal tract with eosinophils, peripheral blood eosinophilia which may be persistent, recurrent episodes of abdominal pain, nausea, vomiting and diarrhea. The syndrome in man closely resembles that reported in the canine. The disease is often seen in young dogs 1-4 years of age and German Shepherds and Cocker Spaniels are the breeds in which the disease has been reported most frequently (7, 35, 53). The symptoms reported in man nearly parallel those reported in dogs. In this species, the disease is characterised by vomiting, diarrhea and leukocytosis with persistent eosinophilia (1). The diagnosis in man

is based on the history, clinical and pathological findings, radiographic evidence of thickened foci in the stomach and small intestine (94) and the presence of persistent eosinophilia. Confirmation of the diagnosis is done through histological examination of biopsy sections of the gastrointestinal tract and associated lymph nodes. The findings in histological examination of the biopsy sections will invariably reveal pronounced tissue eosinophilia. These criteria for human diagnosis have also been employed in the dog (1, 94).

The diagnosis of the disease is generally done when the possibility of intestinal parasitic involvement could be ruled out in man and the dog. Hayden and Van Kruiningen (53, 54) have however reported five cases of confirmed eosinophilic gastroenteritis in German shepherds which gave an indication of the possible involvement of visceral larva migrans. These investigators based their conclusion of the possible helminth involvement in the cause of the disease on the following: (a) All 5 dogs in the report had lesions that were similar in appearance and distribution to those observed in experimentally induced and naturally-occurring infections with Toxocara canis in dogs and mice and with Toxocara cati in cats; (b) One of the five dogs had numerous fragmented larvae within the eosinophilic granuloma in the small intestine; (c) Two of the five dogs had an immune response as evidenced by increased serum beta-globulin content and the precipitation of PAS-positive material around the larvae in the tissues. In another experiment, the authors attempted to reproduce the disease in dogs through experimental infection with T. Canis. This experiment, however, failed to prove that eosinophilic gastroenteritis has any association with helminthiasis in the dog. The only

evidence of gastroenteritis in this experiment was found in histological examinations in which foci of eosinophil infiltrations were observed in the walls of the small intestine (54). Other reports on canine eosinophilic gastroenteritis found complete absence of intestinal parasites (7, 35, 52).

Food antigens have been implicated in man as a possible cause of eosinophilic gastroenteritis. Leinbach (81) reported a partial success in the treatment of a human patient with eosinophilic gastroenteritis. In this case, elimination of animal protein from the diet led to a dramatic improvement initially; however, the peripheral blood eosinophilia remained and the patient suffered a relapse despite continued elimination of animal protein from the diet. Dalinka (33) reported another human case that responded to dietary elimination of protein and corticosteroid therapy. Again a relapse occurred with the withdrawal of the corticosteroid therapy. Gariepy et al (41) reported a complete success in the treatment of the disease with dietary elimination of protein. In the dog, however, possible association of the disease with food antigens has not been reported and the dietary eliminations have no beneficial effects in clinical cases of the disease (1).

In human a history of various allergic disorders such as hay fever and asthma, eczema and urticaria often accompany eosinophilic gastroenteritis. Such histories have not been documented in the dog (1).

The treatment of choice in man and dogs for this disease is a prolonged course of corticosteroid. The drug has been effective in treating this disease. The possibility of a relapse should be considered on withdrawal of corticosteroid therapy. Until the underlying cause of the disease is found, this represents the

only method of the treatment of this disease (1, 7, 52, 108).

Eosinophilic Leukemia

There has been a lot of disagreement as to whether eosinophilic leukemia does exist as a disease entity or not. In the human most of these controversies stemmed from the fact that diagnosed cases of the disease were not associated with significant numbers of immature eosinophils in the peripheral blood of these patients. The diagnosis in these cases was based on the finding of marked peripheral blood eosinophilia. There are however other disease states that are also characterised by marked peripheral blood eosinophilia. This feature is thus not unique to eosinophilic leukemia. Eosinophilia with peripheral counts of up to 82% has been reported with intestinal parasitism (20, 28, 104). Cline (28) reported however that the disease may exist as a separate entity. Herbert and Zucker-Franklin (20) reviewed the work of other investigators who reported on cases of a rarely occurring mature cell neutrophilic leukemia in man. The predominance of mature neutrophils in the peripheral blood in this disease state has led to the speculation that such could be the nature of eosinophilic leukemia in man.

Eosinophilic leukemia first came into existence when a Philadelphia chromosome in a patient with peripheral blood eosinophilia,organomegally and diffuse organ infiltration with eosinophils was reported (28). Benvenisti (11) reported on the criteria for the diagnosis of the disease in man. This requires the presence of severe peripheral blood eosinophilia with blast cells above normal in the bone marrow and/or the peripheral blood. The domestic cat perhaps satisfies these requirements for the diagnosis of the disease. Eosinophilic

leukemia has been reported in the cat and the dog. The disease in the dog is extremely rare (111, 104). Clinical pathologic findings in some of the cat cases revealed great leukocytosis with absolute peripheral counts exceeding 100,000 per cu. mm. Neoplastic eosinophilic cells have been observed in the bone marrow, spleen and various other organs of the cat. In one case involving a cat an eosinophil count of 62% was reported (104). Schalm (111) reported the disease in a cat that had total leukocyte count of 136,000 per cu. mm. and a differential eosinophil count of 35%. In another report, Schalm found a total leukocyte count of 200,000 in a cat with a differential eosinophil count of 80-85%. In this latter report few eosinophilic metamyelocytes were present in the peripheral blood; eosinophilic bands contributed 14-20% of the total differential leukocyte count; mature eosinophils predominated in the bone marrow and the peripheral blood; some of the marrow eosinophilic myelocytes were larger than normal and retained deep blue cytoplasm; there was also a bone marrow hyperplasia with 82% of the cells belonging to the eosinophilic series. At necropsy, in this latter case, there were enlargement of all the thoracic and abdominal lymph nodes and hepatosplenomegally Impression smears of the lymph nodes, liver, spleen and bone marrow revealed many eosinophils and other granulocytic precursor cells. These cases have been presented to demonstrate the variabilities of the disease in the cat with respect to clinical pathological findings. The criteria for the diagnosis of eosinophilic leukemia put forward by Benvenisti (11) thus fit quite well with the findings in the cat. Perhaps the clinical and pathological manifestation of the disease in man takes other forms.

There are a host of other disease conditions in man and animals associated

with eosinophilia. These need to be considered each time cases of eosinophilia are presented, especially in those that show no response to routine therapeutic regimens. It is not considered appropriate to treat each of these diseases separately. References to these disease conditions have been given earlier in both man and animals (28, 104). Of all the domestic animals, the dog and the cat have disease spectra with eosinophilia, that matches those in the human. Eosinophilic ulcerative colitis has been reported in man, dog and cat. The cause of the disease remains unknown but it bears some resemblance to eosinophilic gastroenteritis in man and dog and may have a common cause

Human and animal diseases with eosinophilia are numerous. Research in animal diseases with eosinophilia may eventually shed light on the causes of these diseases in man. Tropical eosinophilia in man had eluded solution for years. The disease was reported in patients returning from the tropics and its cause could not be identified for a long time. Recently, it has been established that Dirofilaria immitis is the etiologic agent. This parasite is also the cause of heartworm disease in the dog (34, 97, 133). This may perhaps serve to indicate that other animal and human diseases associated with eosinophilia may have common causes.

Some Current Advances in Eosinophil Research

Research on the eosinophilic leukocyte has been hampered in many instances due to the fact that the cell is normally present in small numbers in the peripheral blood. This makes it extremely difficult to obtain adequate numbers of the cell for the various studies. For this same reason, it is also difficult to

obtain pure eosinophil fractions, free from contamination by other leukocytes. The inconsistencies in experimental findings in the studies of the cell could partially be attributed to contaminations. In an attempt to overcome some of these problems, investigators have utilized eosinophils from patients with eosinophilia due to variable causes. Perhaps the eosinophils from such subjects were altered in some way by the underlying disease processes. With such state of affairs, experimental results may not be reproducible if these variables are not considered. This then calls for a search for methods of obtaining adequate and reliable eosinophil fractions with known variables that could be standardized and controlled. References, relevant to the theme of this study, that reported on methods of production of peripheral blood eosinophilia include: Archer (4); Robert and Dorothea (112); and Robert and Dorothea (113).

Agar Culture Studies of the Eosinophils

Zucker-Franklin and George (138) reported on the "identification of eosinophil colonies in soft agar cultures by differential staining for peroxidase." This facilitated the study of certain aspects of the functions of the eosinophil in soft agar culture media. This method thus overcame the problems of contamination of eosinophil fractions as well as providing adequate numbers of the cell for the various studies in vitro.

Anti-eosinophil Serum Production

Mahmoud et al (7) described the production of monospecific rabbit

anti-human eosinophil sera. This study for the first time demonstrated the antigenic specificity of the eosinophilic leukocyte among other polymorphonuclear leukocytes. The authors also described the production of anti-mouse eosinophil sera. Tests of these anti-sera showed that there were no cross reactions with the neutrophils.

The human eosinophil antisera were produced from eosinophils obtained from patients with Hodgkin's disease and leukemia. Purified eosinophil fractions were injected into rabbits and the rabbit anti-human eosinophil sera were obtained from the sera. An interesting observation in the patients with Hodgkin's disease was that sera from these patients contained immunoglobulins that were cytotoxic to eosinophils and were also capable of blocking the effect of the antiserum prepared against the patients own eosinophils.

The production of the monospecific anti-eosinophil sera may enhance studies on the functions of the cell. The anti-eosinophil sera may find application in the study of various disease conditions associated with the cells. The specificity of the anti-eosinophil sera may find clinical application in the treatment of diseases such as in parasitosis, allergies and eosinophilic pneumonias. Kay (73) used anti-eosinophil sera in his experiment which demonstrated the role of the eosinophil in parasitic diseases with reference to Schistosoma infections.

Surface Features of the Eosinophil

Szekeres and Szabo (120) conducted an experiment with the leukocytes of patients with immediate type hypersensitivity reactions, using emigrated skin-

window eosinophils. Their findings indicated that these emigrated eosinophils could be conjugated with FITC-labelled anti-human horse globulin. They next attempted to conjugate FITC-labelled anti-human horse globulin with peripheral blood eosinophils but were unsuccessful. This then demonstrated that there are variations in the surface properties of emigrated and circulating eosinophils from the same subject. Past reports indicated that monocytes and macrophages have receptors for IgM, IgG and the first four components of complement. On the basis of this report, it was suggested that the eosinophils may also have receptors for immunoglobulins. Rabellino (106) reported identifying IgG receptors on the eosinophil.

Polliac et al (103) examined the surface architecture of blood eosinophils of patients with non-leukemic eosinophilia, under the scanning electron microscope (SEM). Their findings indicated that under the scanning electron microscope, the surface of the eosinophils was characterised by variable numbers of microvilli; certain proportion of the eosinophils had ridge-like profiles while others had blebs. Similar findings were also reported in an earlier study of the lymphocytes. The significance of this study was that it represented the first report on the surface architecture of the eosinophils under the scanning electron microscope. Secondly, this finding suggests the precautions that need to be taken when examining leukocyte populations under the scanning electron microscope.

Methods of Quantitating Eosinophils in Body Compartments

Routine methods of quantitating the eosinophils in the peripheral blood and

the bone marrow have been provided elsewhere (20, 21, 59, 111). Quantitative studies of eosinophils in the tissues of the body have been reported infrequently.

Litt (83) studied eosinophils in the lymph node of the guinea-pig following an antigenic stimulation. Histological examination by the author revealed that the eosinophils tended to be arranged in clusters with areas studded with only a few or completely devoid of the cells. This led to the realization that the eosinophil numbers in this organ cannot be estimated on the basis of the average number of cells in several adjacent or randomly selected fields. The author then devised a method of quantitating the eosinophils in order to overcome the problem of this erratic distribution. In his method, he scanned completely through a median section, approximately through the hilum of the lymph node and identified fields with the greatest concentration of eosinophils. The cells in these fields were then counted under x 500 magnification (40 objective x 12.5 eyepiece) and the mean of the three highest counts was taken as an index of the eosinophil concentration in the lymph node section. The author reported that the counts obtained were fairly reproducible by different workers with variations lying within 20%. Simultaneous countings of the same field by two observers showed a variation of less than 5%.

Binder (14) reported a quantitative method of studying cell density in the lamina propria of the colon in subjects with ulcerative colitis. His biopsy sections were stained with hematoxyline-Eosin while other were stained with May-Grünwald. He used a grid containing 16 squares with sides measuring 2.5 mm. The grid was inserted into the ocular of the microscope. The slides were

examined under x 100 magnification (100 objective x 10 eyepiece) using an oil immersion lens. Only the squares in the grid containing lamina propria were considered suitable for counting. Squares partly occupied by epithelial cells were excluded. Sections of the mucosa was examined until 128 squares had been counted. Sections with variable cell densities in the different parts were examined the same way, repeating the counting at least twice and then taking the average for 128 squares. The counts were then converted to the "number of cells per mm^2" of lamina propria.

The author (Binder) was able to identify the tissue eosinophils with ease, however it was not possible to differentiate between the other leukocytes with accuracy. For this reason, a differential cell count could not be obtained; however, the number of eosinophils per 1000 cells was obtained. Two countings of 1000 cells each was obtained and the differential tissue count percentage was calculated.

Vilpo (126) also reported a method of quantitating tissue eosinophils. His method was similar to that of Binder. His eosinophil counts were done under x 850 magnification. Two hundred fields were counted and the values obtained were converted to the "number of eosinophils per mm^2."

Archer (4) counted tissue eosinophils under x 400 magnification and reported that the finding of more than 3 or 4 eosinophils under this magnification, per high power field, was indicative of a pathologic condition.

MATERIALS AND METHODS

A total of 16 adult dogs of both sexes were used for this study: all of them were strays impounded in the Manhattan and Junction City areas and were supplied by the Animal Resource Facility of Kansas State University and the Department of Surgery and Medicine, Manhattan, respectively. Eight of these dogs were conditioned at the Animal Resource Facility. The other eight fell into the category of parasitised, as evidenced by the presence of hookworm and roundworm eggs in the feces. Six of the parasitised dogs were obtained from the Animal Resource Facility and the remaining two were supplied by Dykstra Veterinary Hospital. All the parasitised dogs were normal on physical examination except for the evidence of intestinal parasites on fecal examination.

Conditioning of Normal Dogs

Stray dogs selected for research purposes were subjected to routine procedures in the process of conditioning at the Animal Resource Facility. Physical and laboratory examinations were done and treatments were given when necessary. All dogs were routinely wormed with dichlorvos. The selected dogs for this study were further screened critically and wormed to ensure complete absence of intestinal parasites. Final fecal flotation tests were done and found negative a few days before the dogs were obtained. The dogs had received vaccinations for distemper, Hepatitis and Leptospirosis earlier on. Serum samples were submitted for the SMA_{12} tests and were reported normal.

Housing and Feeding of Conditioned Dogs

The conditioned dogs were housed in clean, properly-sized cages and fed dog chow and water for 3 days before the commencement of sampling. The 3 days holding was designed to stablize these dogs.

Parasitised Dogs

The parasitised dogs were selected on the basis of the presence of hookworm and ascarid eggs in the feces, with the fecal flotation tests. Three of the six dogs obtained from the Animal Resource Facility were housed overnight in properly-sized cages and fed only water. The other 5 dogs were obtained at a time that required no housing.

Characteristics of Parasitised Dogs

No.	Breed	Sex	Estimated Age	Weight (kg)
1	Mixed	Female	Adult	15.9
2	Mixed	Male	Adult	13.6
3	Mixed	Male	Adult	27.2
4	Mixed	Female	Adult	21.8
5	Mixed	Male	Adult	20.9
6	Irish Setter, Mixed	Male	Adult	15.0
7	Mixed	Female	Adult	11.4
8	Mixed	Male	Adult	31.8

Characteristics of Conditioned Dogs (Normal)

No.	Breed	Sex	Estimated Age	Weight (kg)
1	Terrier, Mixed	Male	Adult	11.4
2	Schnauzer, Mixed	Female	10 months	9.0
3	Cocker Spaniel	Female	Adult	13.6
4	Dachshund-Terrier	Male	Adult	10.5
5	Mixed	Female	Adult	10.9
6	Mixed	Female	1 year	10.9
7	Spaniel, Mixed	Female	Adult	11.8
8	Spaniel, Mixed	Male	Adult	11.4

Method of Sampling

Half the dogs in each group were sampled in the mornings between 8 a.m. and 10 a.m. The other half were sampled in the afternoons between 1 p.m. and 3 p.m.

Blood samples were obtained from the cephalic vein of each dog and dispensed into EDTA tubes. All blood samples were stored at 4°C for 1-2 hours before processing.

Tissue samples were obtained under general anesthesia prior to euthanasia. The dogs were atropinised and anesthesised with Pentobarbital. A ventral median incision was made into the abdominal cavity to obtain biopsies. Two biopsies were obtained each, for the duodenum, jejunum and ileum and immediately placed into sample bottles containing 10% BNF. Biopsies were also obtained of the mesenteric and bronchial lymph nodes and the bronchus and were also placed in 10% BNF.

After euthanasia, the entire gastrointestinal tract was critically examined for the presence of intestinal parasites.

All the biopsy sections were fixed in the 10% BNF for variable periods ranging from 1-4 weeks prior to submission for histopathological processing.

Hematologic Procedure

For each blood sample, the total leukocyte count, absolute eosinophil count and the differential eosinophil counts were obtained. Blood smears for differential counts were made using "LARC^{TM}/Spinner" (Corning Company). The staining was done with "Hematek slide stainer" (Ames) using Wrights stain.

Preparation of Diluting Fluid for Eosinophil Count

Stock solution was prepared by dissolving 1 gram of "eosin y" in 100 ml of distilled water to make a 1% solution. This solution has been reported stable for a long period of time. From this stock solution, the working solution was prepared as required by adding 0.5 ml of this to 0.5 ml of Acetone and 9.0 ml of distilled water, making a total of 10 ml of working solution. Reports indicated that the working solution may be stable for 2 months. In this study however, fresh working solution was used for no longer than one week.

Preparation of Diluting Fluid for Total Leukocyte Count

N/10 Hydrochloric acid was prepared by adding 0.1 ml of concentrated acid to 10 ml of distilled water and then mixed thoroughly for 3 minutes. This diluting fluid was used for no longer than 3 weeks.

Differential Eosinophil Count

Standard procedure (111) was used for the eosinophil differential count. Wright's stained blood smears were obtained and 200 leukocytes were counted. The percentage of the eosinophils in the smear was then calculated.

Procedure for Absolute Eosinophil Count

1. Two leukocyte diluting pipettes were filled with well mixed blood to the 1 mark, then both were filled with the working "eosin y" solution to the 11 mark making a dilution of 1:10. The mixture was agitated well for 1 minute.

2. Three drops were discarded from each pipette and the Fuchs-Rosethal counting chamber was filled on both sides then placed in a moist chamber for 15 minutes.

3. Using x 100 magnification, eosinophils were counted in all 9 squares on both sides of the counting chamber and the average for 9 squares was obtained.

4. Total number of eosinophils per cu. mm. = average number of cells per 9 squares x 11.1.

Procedure for Total Leukocyte Count

1. Well mixed blood was drawn into a leukocyte pipette to the 0.5 mark.

2. The pipette was filled with N/10 HCI (working solution) to the mark 11.

3. Three drops were discarded and both sides of the counting chambers were filled.

4. The leukocytes were allowed to settle for 1 minute, then the cells in

each of the four corner squares were counted on both sides of the counting chamber under x 100 magnification.

5. The average number of cells for the 4 corner squares was obtained.

6. $\text{Wbc/cu.mm} = \dfrac{\text{Number of cells x 20 x 10}}{4}$

Histological Procedures
for the Tissues

The biopsy tissues were submitted in sample bottles containing 10% BNF. These were trimmed to approximately 2 cm square x 4 mm thickness and properly identified. Each piece of tissue was placed in a metal cassette with the identification and returned to 10% BNF to await processing.

Each tissue was then dehydrated, cleared and infiltrated using the Autotechnicon machine. The machine contains 12 solutions and the tissues were timed to remain in each solution for 1 hour. The tissue moves from 10% BNF through two changes each of 80% alcohol, 95% alcohol, and absolute alcohol. This was then cleared with xylene (two changes of xylene and two changes of paraffin). The complete infiltration with paraffin was obtained using a vacuum infiltrator oven for 15-20 minutes. The tissues were then embedded in paraffin to produce blocks that will perfectly fit the microtome. The paraffin embedded sections were cut with rotary microtome to a thickness of 6 microns. The sections were then placed on slides and de-paraffinized, then placed in four changes of xylene for 2 minutes each. The sections were then hydrated through two changes of 100% alcohol, one change each of 95%, 80% and 60% alcohol then finally into distilled water. Staining was done for 5 minutes with hematoxylene-eosin after which they were mounted on

glass slides.

Quantitation of Tissue Eosinophils

The number of tissue eosinophils were counted per high power field for the duodenum, jejunum and ileum. The ocular micrometer was used in the ocular of the microscope to aid in the counting. For these sections, only the eosinophils in the subvillus laminar propria were counted. Fields containing epithelial structures were not considered suitable for counting. A total of 10 fields were counted under x 400 magnification (40 objective x 10 eyepiece). The entire section was then examined under x 200 magnification (20 objective x 10 eyepiece) and the total number of eosinophilic granulomas was obtained.

For the lymph nodes, similar countings were also obtained. The eosinophils in the subcapsular and the cortical regions of the mesenteric and bronchial lymph nodes were counted per high power field. The total number of eosinophilic granulomas was similarly obtained. The medullary regions of the lymph nodes in this study often contain misleading eosinophilic precipitates of unknown origin and for this reason, this area was not included in the counting.

For the bronchi, the eosinophils in the lamina propria of the tunica mucosa were counted in the same manner under x 400 and x 200 magnifications.

Recording of Counts

The total number of eosinophilic granulomas were recorded in absolute numbers per section. The other grading was based on the average number of eosinophils per high power field under x 400 magnification and these were recorded

as "eosinophil cell densities" on a scale of 0–4 (see Table A below).

Average number of eosinophils/Hpf	Density Grading
0 – 5	0
6 – 15	+1
16 – 25	+2
26 – 35	+3
> 35	+4

RESULTS

Statistical analysis was done and revealed significant differences between the parasitised and non-parasitised groups of dogs in this study. The interactions between the various quantitative measurements in the two groups with respect to the time of sampling was also computed. Student's t test and the chi-square test were employed for this analysis. Blood and tissue granuloma data were processed by computer. Tissue eosinophil density was processed manually due to the small number of quantitative measurements involved. Raw data have been tabulated in the appendix.

Statistical Analysis for Eosinophilic Granuloma and Absolute Eosinophil Count Data

Preliminary "unequal subclass analysis of variance" was computed for the parasitised and non-parasitised groups of dogs to obtain the means and variances. From these, the analysis of the variances for the duodenum, jejunum, ileum, mesenteric lymph node, bronchus bronchial lymph node and the absolute eosinophil counts were then computed. These preliminary analyses then provided the basis for the final statistical analysis and interpretation of the data for the two groups of dogs in this study.

Table 1: Means and standard deviations for tissue eosinophilic granuloma and absolute eosinophil counts for parasitised and non-parasitised groups of dogs.

Type of Tissue	Parasitised No. of Granuloma and Absolute Eos. Counts	Non-Parasitised No. of Granuloma and Absolute Eos. Counts	Statistical Significance
Duodenum	16.778 (SD 0.15)	1.083 (SD 0.15)	*
Jejunum	18.021 (SD 0.16)	3.608 (SD 0.16)	*
Ileum	22.701 (SD 0.63	3.809 (SD 0.63	*
Mesenteric L.N.	20.047 (SD 0.64)	3.503 (SD 0.64)	*
Bronchus	1.748	0.0	-
Bronchial L.N.	11.157 (SD 0.06)	3.772 (SD 0.06)	*
Absolute Eosinophil Counts/mm^3	682.0 (SD 9.10)	635 (SD 9.10)	NS

* = Statistically Significant ($p. < .05$)

\- = No Analysis - Insufficient Data

NS = Not Statistically Significant

Table 2: Mean and standard error of tissue granuloma and absolute eosinophil counts data with reference to the time of sampling for parasitised and non-parasitised groups of dogs.

Type of Tissue	Time of Sampling		Statistical Significance
	Morning	Afternoon	
Duodenum	2.295 (0.391)	2.843 (0.391)	NS
Jejunum	3.001 (0.876)	3.132 (0.876)	NS
Ileum	3.092 (0.796)	3.624 (0.796)	NS
Mesenteric L.N.	3.514 (0.802)	2.835 (0.802)	NS
Bronchus	0.610 (0.226)	0.712 (0.226)	-
Bronchial L.N.	2.129 (0.237	2.553 (0.237)	NS
Absolute Eosinophil Counts/mm^3	27.249 (3.037)	24.090 (3.037)	NS

NS = Not Statistically Significant
\- = No Analysis - Insufficient Data
( ) = Standard Error

Table 3: Interactions within the various quantitative measurements of tissue granuloma and absolute eosinophil counts with reference to the time of sampling for parasitised and non-parasitised groups of dogs (means and standard errors).

Type of Tissue	Time of Sampling						Statistical Significance
	Morning			Afternoon			
Duodenum	P	3.553	(0.553)	P	4.641	(0.553)	
	NP	1.036	(0.553)	NP	1.045	(0.553)	NS
Jejunum	P	3.500	(1.238	P	4.991	(1.238	
	NP	2.515	(1.238	NP	1.274	(1.238	NS
Ileum	P	4.512	(1.125)	P	5.017	(1.125)	
	NP	1.671	(1.125)	NP	2.232	(1.125)	NS
Mesenteric L.N.	P	4.845	(1.134)	P	4.109	(1.134)	
	NP	2.183	(1.134)	NP	1.561	(1.134)	NS
Bronchus	P	1.219	(0.320)	P	1.425	(0.320)	
	NP	0.0	(0.320)	NP	0.0	(0.320)	-
Bronchial L.N.	P	3.376	().335)	P	3.304	(0.335)	
	NP	2.083	(0.335	NP	1.801	(0.335)	NS
Absolute Eosinophil Count/mm^3	P	25.421	(4.295	P	26.830	(4.295)	
	NP	29.076	(4.295)	NP	21.350	(4.295)	NS

SS = Statistical Significance
NS = Not Statistically Significant
\- = No Analysis - Insufficient Data
P = Parasitised
NP = Non-Parasitised
( ) = Standard Error

Table 4: Analysis of tissue cell density for parasitised and non-parasitised groups of dogs.

Tabulations were based on the formula: $E = \frac{RC}{N}$

E = Expected
R = Sum of frequencies in each row
C = Sum of frequencies in each column
N = Total number of observations

Expected values for tabulation are placed in the small rectangles.

The equivalent observed values are in the larger rectangles.

Calculations or final result of the analysis are placed to the right of each tabulation for each tissue type.

Table 4.1: Duodenum

	Non-Par.		Par.		
0	5	3	1	3	6
+1	3	1.5	0	1.5	3
+2	-	1.5	3	1.5	3
+3	-	1	2	1	2
+4	-	1	2	1	2
	8		8		16

Calculations

$X^2c = 12.70$

$X^2.05^{(4)} = 9.49$

$X^2.025^{(4)} = 11.14$

$X^2.01^{(4)} = 13.28$

$X^2.005^{(4)} = 14.86$

$.01 < \hat{\alpha} < .025$

Table 4.2: Jejunum

	Non-Par.		Par.		
0	5	3	1	3	6
+1	2	1	-	1	2
+2	1	2	3	2	4
+3	-	-	-	-	0
+4	-	2	4	2	4
	8		8		16

Calculations

$X^2c = 9.7$

$X^2.05^{(3)} = 7.81$

$X^2.025^{(3)} = 9.35$

$X^2.01^{(3)} = 11.34$

$X^2.005^{(3)} = 12.84$

$.01 < \hat{\alpha} < .025$

Table 4.3: Ileum

	Non Par.		Par.		
0	4	2	0	2	4
+1	3	2	1	2	4
+2	-	1	2	1	2
+3	-	1	2	1	2
+4	1	2	3	2	4
	8		8		16

<u>Calculations</u>

$X^2_c = 10.0$

$X^2_{.05}(4) = 9.49$

$X^2_{.025}(4) = 11.14$

$X^2_{.01}(4) = 13.28$

$X^2_{.005}(4) = 14.86$

$.025 < \hat{\alpha} < .05$

Table 4.4: Mesenteric Lymph Node

	Non Par.		Par.		
0	2	1	-	1	2
+1	1	.5	-	.5	1
+2	3	1.5	-	1.5	3
+3	1	1	1	1	2
+4	1	4	7	4	8
	8		8		16

<u>Calculations</u>

$X^2_c = 10.50$

$X^2_{.05}(4) = 9.49$

$X^2_{.025}(4) = 11.14$

$X^2_{.01}(4) = 13.28$

$X^2_{.005}(4) = 14.86$

$.025 < \hat{\alpha} < .05$

Table 4.5: Bronchial Lymph Node

	Non Par.		Par.		
0	-	-	-	-	-
+1	6	3	-	3	6
+2	-	.5	1	.5	1
+3	1	1	1	1	2
+4	1	3.5	6	3.5	7
	8		8		16

<u>Calculations</u>

$X^2 = 12.0$

$X^2_{.05}(3) = 7.81$

$X^2_{.025}(3) = 9.35$

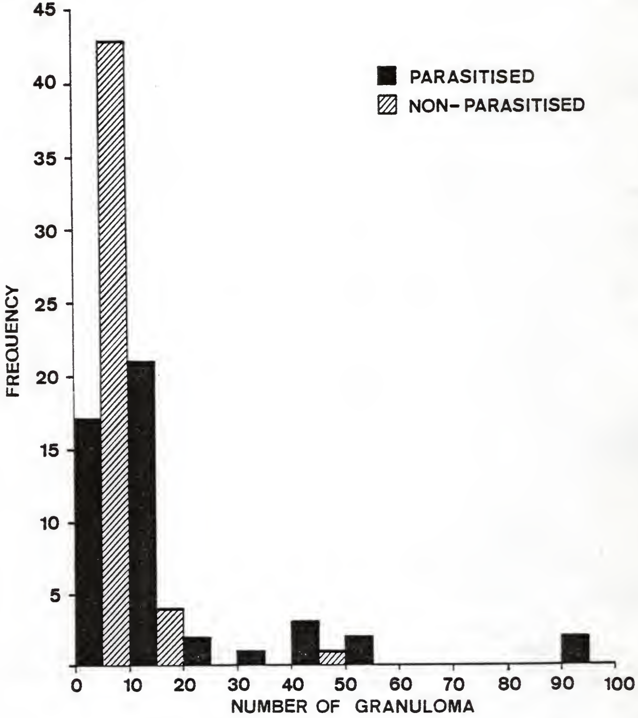
$X^2_{.01}(3) = 11.34$

$X^2_{.005}(3) = 12.84$

$.005 < \hat{\alpha} < .01$

HISTOGRAM

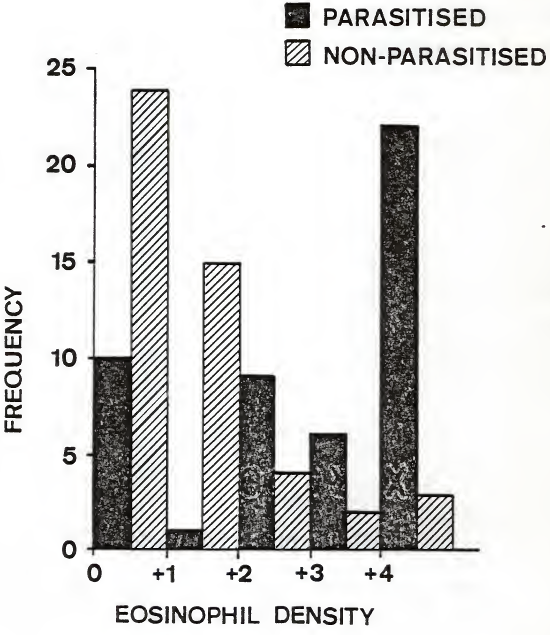
Figure 3.1: Frequency Distribution of Eosinophilic Granuloma



45
40
35
30
25
20
15
10
5
FREQUENCY
PARASITISED
NON-PARASITISED
0
10
20
30
40
50
60
70
80
90
100
NUMBER OF GRANULOMA

HISTOGRAM

Figure 3.2: Frequency Distribution of Tissue Eosinophil Density


PARASITISED
NON-PARASITISED
25
20
15
10
5
0
FREQUENCY
0
+1
+2
+3
+4
EOSINOPHIL DENSITY

Interpretation of Data Analysis

Reference to Table 1 will indicate that there are significant statistical differences between the parasitised and the non-parasitised groups of dogs with respect to the number of eosinophil granulomas in the tissues examined ($P < .05$). The means of the tissue granulomas were higher in the parasitised group of dogs than in the non-parasitised. This table (1) also revealed that in the gastrointestinal tract tissues examined, the highest number of eosinophil granulomas was located in the ileum of both parasitised and non-parasitised groups of dogs with a mean of 22.70 and 3.81 respectively. The second highest eosinophil granuloma concentration in the gastrointestinal tract was located in the jejunum with a mean of 18.02 for the parasitised group and a mean of 3.61 for the non-parasitised. The lowest count of eosinophil granuloma was recorded in the duodenum with a mean of 16.78 and 1.08 for the parasitised and the non-parasitised groups, respectively. The mesenteric lymph node has a relatively higher number of eosinophil granuloma when compared with the bronchial lymph node in the parasitised dogs. A mean of 20.05 was recorded for the mesenteric lymph node against 11.16 in the bronchial lymph node for the parasitised group of dogs. In the non-parasitised group, however, the number of eosinophil granuloma in the mesenteric and bronchial lymph nodes was about the same, with a mean of 3.50 and 3.77, respectively. The bronchus showed no significant eosinophil granuloma concentration and was thus dropped from further analysis. Fig. 3.1 (appendix) and the accompanying histogram was prepared from raw data to show the frequency distribution of eosinophil granuloma in the parasitised and non-parasitised groups of dogs. The last

recording from Table 1 showed no statistically significant difference between the parasitised and the non-parasitised dogs with respect to the absolute peripheral eosinophil count. A mean of 682.0 and 635.0 was recorded for the parasitised and the non-parasitised dogs, respectively.

Table 2 data were generated to determine variations in the number of eosinophil granulomas and absolute eosinophil counts with respect to the time of sampling (morning and afternoon) for the two groups of dogs. The table has shown that there were no significant statistical differences in samples drawn in the mornings or in the afternoons. Again statistical analysis on this parameter had been dropped for the bronchi for reasons cited earlier.

Data for Table 3 were generated to demonstrate the interactions between various quantitative measurements in the two groups of dogs with regards to the time of sampling. The statistical analysis indicated no significant interactions between these measurements in the two groups of dogs. This implies that there was no relationship between eosinophil granuloma and absolute eosinophil count with the time of sampling (morning or afternoon) for the parasitised and non-parasitised groups of dogs. The other implication is that, within each group, there were no significant differences between samples in the morning and afternoon samples.

Data for Table 4 were generated to show the differences in the tissue eosinophil cell densities in the parasitised and the non-parasitised groups of dogs. Reference to these tabulations (4.1; 4.2; 4.3; 4.4; and 4.5) indicates the same trend as observed with the measurements of eosinophil granuloma.

These tabulations and calculations indicated a significant statistical difference in tissue eosinophil cell density between the parasitised and the non-parasitised groups of dogs with a higher eosinophil density in the parasitised than in the non-parasitised dogs. Analysis for the bronchi has not been tabulated for reasons cited earlier. The findings in the statistical analysis for eosinophil granuloma also seem to hold true for the eosinophil cell densities. There was no relationship between the eosinophil cell density and the absolute peripheral eosinophil counts, irrespective of the time of sampling. Fig. 3.2 and the corresponding histogram show the frequency distribution of tissue eosinophil densities for the parasitised and the non-parasitised groups of dogs.

Finally, Fig. 4.1 was presented to provide a comparison of morning and afternoon absolute peripheral eosinophil counts in the two groups of dogs, in order to support earlier statistical conclusions.

DISCUSSION

A distinct statistically significant difference was found in tissue eosinophil semi-quantitative measurements between the parasitised and non-parasitised groups of dogs in this study. Both tissue eosinophil density and total number of eosinophilic granulomas were found to be significantly higher in the parasitised dogs than in the non-parasitised. These findings were true irrespective of the sampling time.

Table 1 shows that the highest concentration of eosinophilic granuloma in the parasitised group of dogs was located in the ileum with a mean of 22.70. The jejunum had the second highest number of granulomas with a mean of 18.02 and the duodenum was rated last with a mean of 16.78. The general pattern thus indicates a progressive increase in the numbers of eosinophilic granulomas from the duodenum to the ileum in the parasitised group of dogs. In the non-parasitised group, however, there was almost no difference between the granuloma content of the ileum and jejunum (means of 3.81 and 3.61 respectively). The duodenum in this group also displayed the least number of eosinophilic granulomas (mean 1.08).

In the lymph nodes, also, the parasitised dogs significantly dominated over the non-parasitised in both granuloma content and eosinophil density. In the parasitised dogs, the mesenteric lymph node had a higher incidence of eosinophilic granulomas than the bronchial lymph node with means of 20.05 and 11.15, respectively. There seems to be a direct relationship between the incidence of

eosinophilic granulomas within the gastrointestinal tract and the associated mesenteric lymph node. This same relationship appears to be true also with regards to eosinophil density measurements between these organs. The direct relationship between eosinophil densities in the gastrointestinal tract and the associated mesenteric lymph node seems to support the view that the eosinophils within the walls of the intestines could also be drained through the lymphatics into the mesenteric lymph nodes, where they are finally destroyed. This relationship was, however, not observed between the bronchus and the bronchial lymph node in this study. The eosinophil within the bronchi in both groups of dogs were found to be insufficient for any statistical analysis.

Attempts to find any relationship between blood and tissue eosinophil measurements were unsuccessful in this study. No relationship was found between the absolute peripheral eosinophil count and tissue eosinophilic granuloma or density in both groups of dogs. Figures 1.1, 1.2, 1.3, 1.4, and 1.5 demonstrate this fact. A relationship between absolute peripheral eosinophil count and tissue eosinophilic granuloma was not expected since this latter is a measure of chonicity. Granulomas represent sites of antigen localization capable of prolonged attraction of peripheral blood eosinophils. Most disease conditions associated with peripheral blood eosinophilia are also accompanied by infiltration of the tissues with eosinophils. In this respect a relationship between the absolute peripheral eosinophil count and the tissue eosinophil cell density may be expected. Such relationship may, however, be affected by variables such as the diurnal variations of the absolute peripheral eosinophil counts.

With blood to tissue eosinophil ratio of 1:300 and with the tissue eosinophil life-span of approximately 8–12 days, it seems most unlikely that the relationship of the blood and tissue eosinophil numbers could be deduced through a single sampling. The findings in this study were thus appropriate on the basis of established facts. The sampling procedure required to reveal this relationship needs further investigation.

The findings in this study indicate that there was no statistically significant difference in the peripheral absolute eosinophil counts between the parasitised and the non-parasitised groups of dogs. Further, the findings indicate that within each group, there was no significant difference between blood samples drawn in the morning and afternoon. The association of eosinophilia with intestinal parasitism has been treated elsewhere in this report. In this respect, the general inclination has been towards the expectation of increased numbers of circulating eosinophils with parasitism. When viewed on the surface, the findings in this study on the absolute peripheral eosinophil count seem to contradict earlier reports. Based on the clinical orientation of this study, a logical and probably a beneficial explanation could be offered to explain these blood findings. Considering the size of our sample the outcome of some of these findings could not be over emphasised. Table 1 has indicated that the mean absolute peripheral eosinophil counts in the parasitised and non-parasitised dogs were 682.0 and 635.0 per cu. mm respectively. Figure 4.1 (see appendix table) shows the actual figures recorded for the two groups, arranged in ascending order. The figure further indicated that the highest absolute peripheral eosinophil counts of

1643 and 1421 per cu. mm. were both recorded within the parasitised dogs in the morning and afternoon blood samples, respectively. Whether some of these absolute peripheral counts lie within normal or abnormal range, depends on individual interpretation. Schalm (111) has reported a normal range of 100-1250 cells per cu. mm. in the dog. The author further cited works which indicated variations in these counts with age. In this study, therefore, it was apparent that most of the absolute peripheral eosinophil counts in both groups of dogs supposedly lie within the normal range established by Schalm.

Blood samples for the absolute eosinophil counts were drawn between 8 a.m. and 10 a.m. in the morning and between 1 p.m. and 3 p.m. in the afternoon. These sampling times thus provided an interval of approximately 5 hours between morning and afternoon samples. Dirunal variations in the absolute peripheral eosinophil counts in the various species have been reported earlier. These reports have been controversial due to the variabilities. Saran and Sanyal (110) have cited works that reported finding lowest absolute counts between 9-11 a.m. and the highest counts around midnight in the human. Schalm (111) reported that in the dog, the morning absolute peripheral eosinophil counts were relatively higher than those in the afternoons. Halberg et al (51) also reported that the lowest peripheral counts occur in preparation for daily activities. In this respect, the morning counts will be expected to be relatively higher than the afternoon counts in the mammalian species, other than the nocturnal animals. The general inference of these reports supports the view that high counts correspond with periods of least physical activity or around midnight in the non-nocturnal

species and vice versa.

Hayden and Van Kruiningen (53) have demonstrated in an experimental infection of the dog with Toxocara canis that absolute peripheral eosinophil counts up to 12,000 cells per cu. mm. could be obtained with intestinal helminthiasis. However, this represents superinfection of the dogs with T. canis. In our study, however, the parasitised dogs were selected on the basis of finding appreciable numbers of hookworm and/or ascarid eggs in fecal flotation tests. The fingind of 20-25 eggs per field at x 200 magnification was taken as the minimum standard for selection. The degree of infestation with intestinal parasites could not be standardized nor accurately estimated in these dogs. Necropsy provided the only means of assessing the degree of parasitism with reasonable certainty. This latter examination revealed fair to moderate degree of infestation with intestinal parasites, consisting mainly of hookworms and few ascarids. From this, it can be seen that the severity of intestinal parasitism as assessed by the fecal flotation tests did not correlate with the degree of infestation revealed at necropsy. However, one of the objectives of this study was to study the eosinophils in natural infestation of the dog with intestinal parasites, as such an approach may more likely have significant clinical application. The inference in the study of the parasitised group of dogs in that although peripheral blood eosinophilia is the hallmark of several disease conditions, including intestinal helminthiasis, the presence of intestinal parasites normally capable of inducing eosinophilia (those with migratory larval stages in the tissues) does not necessarily result in the finding of high absolute eosinophil count in the

peripheral blood.

The failure to demonstrate significant differences in the absolute peripheral eosinophil count between the parasitised and the non-parasitised groups of dogs may be multifactorial; first there is the sampling time to consider. With a sampling interval of approximately 5 hours, it may be hypothesised that to demonstrate the difference between morning and afternoon absolute peripheral eosinophil count and probably between the parasitised and the non-parasitised dogs, this sampling interval should be extended beyond 5 hours. However, since the design of this study was intended to have possible clinical application, these sampling times have been selected to fall within normal daily clinic hours of 8 a.m. to 5 p.m. as well as for convenience in the processing of the blood samples. Secondly, the failure to demonstrate significant difference in the two groups may be related to the degree of infestation with migratory stages of hookworms and ascarids. Perhaps the degree of tissue parasitism by helminth larvae was not high enough to reveal the differences in the absolute peripheral eosinophil counts in the two groups. Considering the fact that migration of helminth larvae is enhanced in immuned dogs, it could be contended that the immune status of our subjects wasn't adequate enough to enhance larval migration which ultimately precipitates peripheral blood eosinophilia. This latter statement, however, needs qualification, considering the findings in tissue eosinophil quantitative measurements. Thirdly, there is a stress factor to consider since the parasitised group of dogs were obtained from stray and were not stabilized prior to blood sampling. This latter approach was chosen to match

equivalent stress factor to which client dogs are exposed within the clinic environment. It could be contended finally that all the above factors may have a significant role in the outcome of the study on the absolute peripheral eosinophil counts in the parasitised and non-parasitised groups of dogs.

CONCLUSION

Comparative study of blood and tissue eosinophils in parasitised and non-parasitised dogs at two sampling times (morning and afternoon) found the following:

1. That there was a statistically significant difference between the parasitised and the non-parasitised groups of dogs with respect to tissue eosinophil cell density and granuloma within the walls of the gastrointestinal tract, the mesenteric and the bronchial lymph nodes.

2. That there was no significant difference between morning and afternoon absolute peripheral eosinophil counts within each group (parasitised and non-parasitised).

3. That there was no significant difference in the absolute peripheral eosinophil counts between the parasitised and the non-parasitised groups of dogs, irrespective of the time of sampling.

4. That there was no relationship between the absolute peripheral eosinophil counts and tissue eosinophil cell density or granuloma, irrespective of the time of sampling.

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APPENDIX

Raw Data Tabulations (Graph)

M = Morning Sampling

A = Afternoon Sampling

Figure 1.1: Duodenum (Parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
9	0	12	433	M
10	+3	19	1421	A
11	+4	10	224	M
12	+2	12	1643	M
13	+2	17	644	M
14	+2	14	810	A
15	+2	12	555	A
16	+4	49	310	A

Figure 1.2: Jejunum (Parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
9	+2	11	433	M
10	+2	8	1421	A
11	+4	59	224	M
12	+2	9	1643	M
13	0	0	644	M
14	+4	10	810	A
15	+4	17	555	A
16	+4	97	310	A

Figure 1.3: Ileum (Parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
9	+2	7	433	M
10	+4	48	1421	A
11	+3	17	224	M
12	+1	16	1643	M
13	+3	53	644	M
14	+4	21	810	A
15	+4	43	555	A
16	+2	4	310	A

Figure 1.4: Mesenteric Lymph Node (Parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
9	+4	93	433	M
10	+3	12	1421	A
11	+4	21	224	M
12	+4	2	1643	M
13	+4	14	644	M
14	+4	31	810	A
15	+4	18	555	A
16	+4	10	310	A

Figure 1.5: Bronchial Lymph Node (Parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
9	+3	11	433	M
10	+4	6	1421	A
11	+2	9	224	M
12	+4	8	1643	M
13	+4	19	644	M
14	+4	10	810	A
15	+4	13	555	A
16	+4	16	310	A

Figure 2.1: Duodenum (Non-parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
1	0	0	777	A
2	0	3	388	A
3	0	6	644	A
4	0	0	1054	M
5	+1	1	1365	M
6	+1	2	755	M
7	+1	3	377	M
8	0	0	155	A

Figure 2.2: Jejunum (Non-parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
1	0	0	777	A
2	0	0	388	A
3	0	6	644	A
4	0	3	1054	M
5	+1	1	1365	M
6	+1	19	755	M
7	0	3	377	M
8	+2	7	155	A

Figure 2.3: Ileum (Non-parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
1	0	1	777	A
2	0	0	388	A
3	+4	48	644	A
4	+1	5	1054	M
5	0	0	1365	M
6	+1	6	755	M
7	+1	4	377	M
8	0	1	155	A

Figure 2.4: Mesenteric Lymph Node (Non-parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
1	0	0	777	A
2	+4	18	388	A
3	0	0	644	A
4	+2	16	1054	M
5	+3	11	1365	M
6	+1	2	755	M
7	+2	0	377	M
8	+2	4	155	A

Figure 2.5: Bronchial Lymph Node (Non-parasitised)

Dog No.	Eosinophil Density	Total No. of Granuloma	Absolute Eosinophil Count	Time
1	+1	2	777	A
2	+1	7	388	A
3	+1	3	644	A
4	+1	1	1054	M
5	+3	5	1365	M
6	+1	7	755	M
7	+4	6	377	A
8	+1	2	155	A

Figure 3.1: Frequency Distribution of Eosinophil Granuloma for Sections Examined in Parasitised and Non-parasitised Groups of Dogs

No. of Granuloma	Parasitised	Non-parasitised
0 - 10	17	43
10 - 20	21	4
20 - 30	2	0
30 - 40	1	0
40 - 50	3	1
50 - 60	2	0
60 - 70	0	0
70 - 80	0	0
80 - 90	0	0
90 - 100	2	0
Total	48	48

Figure 3.2: Frequency Distribution of Tissue Eosinophil Cell Density for Sections Examined in Parasitised and Non-parasitised Groups of Dogs

Eosinophil Density	Parasitised	Non-parasitised
0	10	24
+1	1	15
+2	9	4
+3	6	2
+4	22	3
	48	48

Figure 4.1: Absolute Eosinophil Counts in Parasitised and Non-parasitised Groups of Dogs, Tabulated to Show the Comparison between Morning and Afternoon Recordings in the Two Groups (Figures arranged in ascending order)

Identification	Morning (per cu. mm)	Identification	Afternoon (per cu. mm
11	224 - parasitised	16	310 - parasitised
9	443 - parasitised	15	555 - parasitised
13	644 - parasitised	14	810 - parasitised
12	1643 - parasitised	10	1421 - parasitised
7	377 - non-parasitised	8	155 - non-parasitised
6	755 - non-parasitised	2	388 - non-parasitised
4	1054 - non-parasitised	3	644 - non-parasitised
5	1365 - non-parasitised	1	777 - non-parasitised

STUDY OF BLOOD AND TISSUE EOSINOPHILS IN PARASITISED AND NON-PARASITISED DOGS

by

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ABSTRACT

The investigation was undertaken to compare peripheral blood absolute eosinophil counts and tissue eosinophil cell density and the number of eosinophilic granulomas in parasitised and non-parasitised groups of dogs at two sampling times (morning and afternoon).

Blood samples were obtained in the mornings between 8 a.m. and 10 a.m. and in the afternoon, between 1 p.m. and 3 p.m. for the measurement of the absolute peripheral eosinophil counts.

Tissue samples were obtained at necropsy under general anesthesia, using pentobarbital and biopsies of the duodenum, jejunum, ileum, mesenteric lymph node, bronchus and bronchial lymph node were obtained and immediately placed in sample bottles containing 10% BNF for fixation.

Histopathological sections were obtained using Hematoxylene - eosin stain.

Eosinophil cell density was measured within the subvillus lamina propria of the duodenum, jejunum and ileum of the gastrointestinal tract, the cortical and the subcapsular regions of the mesenteric and bronchial lymph nodes and the lamina propria of the tunica mucosa of the bronchi, under x 400 magnification. The densities of the eosinophils in these tissues were graded 0 to +4 as follows:

1. 0 - 5 cells per high power field = 0
2. 5 - 15 cells per high power field = +1
3. 15 - 25 cells per high power field = +2
4. 25 - 35 cells per high power field = +3
5. $>$ - 35 cells per high power field = +4

All the counts were conducted without knowledge of whether the tissues were from parasitised or non-parasitised dogs.

Eosinophilic granulomas were counted within the mucosa of the duodenum, jejunum, and ileum of the gastrointestinal tract, and the cortical and the subcapsular regions of the mesenteric and bronchial lymph nodes and the lamina propria of the tunica mucosa of the bronchi under x 200 magnification and recorded in absolute numbers.

The findings indicated statistically significant differences between the parasitised and the non-parasitised dogs with respect to tissue eosinophil cell density and the numbers of granuloma. No statistically significant differences were found between the two groups of dogs in the absolute peripheral blood eosinophil counts, irrespective of the time of sampling. There was also no relationship between the absolute peripheral blood eosinophil count and the eosinophil cell density or granuloma, irrespective of the time of sampling.